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Activity of Lactate Dehydrogenase but Not Its Concentration of Messenger RNA Increases With Body Size in Barred Sand Bass, *Paralabrax nebulifer* (Teleostei)

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In white skeletal muscle of conspecific pelagic fishes, the activities of enzymes associated with anaerobic glycolysis, e.g., *lactate dehydrogenase (LDH), usually scale positively with increasing body size; this pattern is opposite to that found for enzymes of aerobic metabolism, which decrease in mass-specific activity with size (1–3). The higher mass-specific capacities for anaerobic ATP generation in larger conspecifics are thought to facilitate conservation of high-speed ("burst") swimming ability in fishes of different sizes (1). To investigate the mechanisms responsible for scaling of LDH activity in white muscle, we quantified LDH activity, total RNA, and the specific mRNA for LDH-A (the skeletal muscle isoform of the enzyme) in white muscle of* Paralabrax nebulifer, *the barred sand bass. We also measured total protein concentration and the concentration of actin, the major protein of thin filaments, and its specific mRNA. Although LDH activity scaled significantly with body size as predicted (1–4), no other biochemical trait measured showed a significant size-dependent concentration. We conclude that the regulation of LDH activity in white muscle of this species is not governed by LDH-A mRNA concentrations, but rather by one or more other mechanisms, for example, an elevated rate of translation of LDH message or a reduced rate of degradation of LDH-A in larger fish.*

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Among pelagic fishes with the ability to swim in strong bursts, conspecifics show large size-dependent changes in muscle enzymatic activity (1–3, 5). When the activities of glycolytic enzymes like LDH and pyruvate kinase, which are critical for ATP generation under oxygen-limiting conditions, are normalized on the basis of units per gram of muscle, they show an increase in larger conspecifics. In contrast, enzymes associated with aerobic respiration, *e.g.*, citrate synthase and cytochrome *c* oxidase, decrease in accord with well-known concepts for scaling of whole-organism aerobic respiration (1–3, 6). The positive scaling of glycolytic enzymatic activities with body size has been interpreted as providing size-independent capacities for high-speed burst swimming, *i.e.*, for sustaining an ability to maintain relative swimming speeds (body lengths s^{-1}) that are identical in different sized individuals of a pelagic species with subcarangiform locomotion (1).

The mechanisms that regulate enzyme concentrations in size-specific patterns are unknown. Clearly, the opposite scaling patterns noted for enzymes of anaerobic *versus* aerobic pathways of ATP generation indicate that regulation of overall protein synthesis or degradation is not a sufficiently specific mechanism to bring about this type of regulation.

To address this issue, we examined size-related biochemical properties of white skeletal muscle of the barred sand bass, *Paralabrax nebulifer*, an active subcarangiform swimmer. Fish were collected by seining on beaches in San Diego, California, and maintained in flowing, ambient seawater at the Scripps Institution of

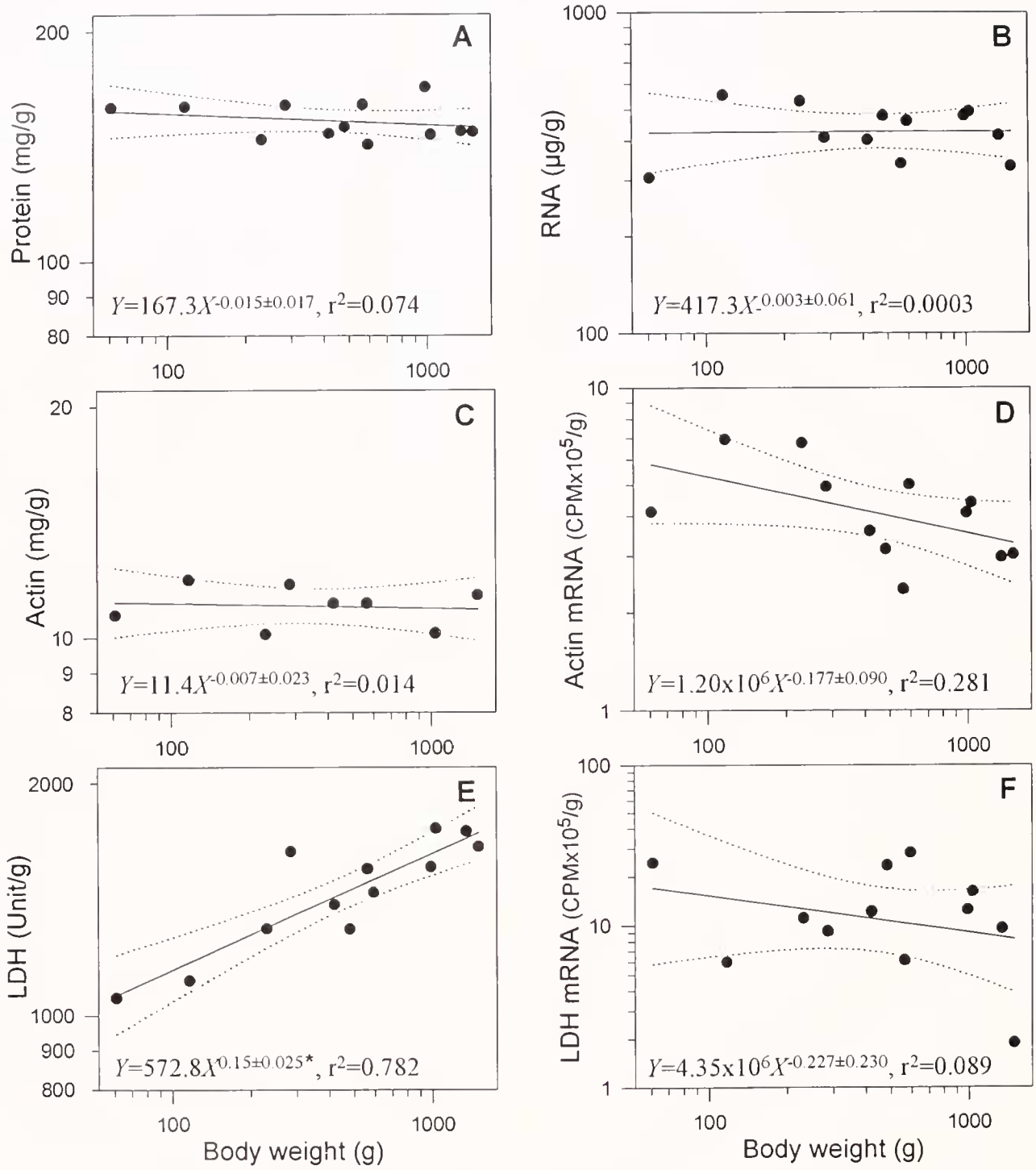

Figure 1. The relationships between body mass and concentrations or activities of proteins and RNAs in the white epaxial muscle of the barred sand bass, *Paralabrax nebulifer*. All the biochemical parameters are expressed as values per gram wet weight, and each dot represents the mean value for duplicate measurements. The equation used is of the general form $Y = aX^b$. Where the slope (b) of the regression line is significantly different from 0 ($P < 0.05$), an asterisk (*) is shown. (A) Muscle total protein concentration as a function of body weight. The total muscle homogenate was used in the bicinchoninic acid assay (13) for determining protein concentration. (B) Muscle total RNA as a function of body weight. The total RNA was determined by measuring a small aliquot of total muscle RNA extract (7) at OD 260. (C) Muscle actin

Oceanography. They were fed *ad libitum* on chopped squid every other day for 3 weeks before sacrifice (by cervical transection). Specimen weights ($n = 12$) ranged from 61 g to 1508 g (Fig. 1). Immediately after sacrifice, two pieces (about 2 g each) of white skeletal muscle were removed from the epaxial white musculature near the base of the first few dorsal spines of the fish. Each tissue sample was quickly minced on a glass plate held at ice temperature and then extracted for RNA, following the protocol of Yang and Somero (7). The rest of the specimen was immediately frozen at −70°C, for later use in measurements of total protein and actin concentration and LDH activity. LDH was chosen as an enzyme with a strong positive scaling pattern (1–4), and actin was selected because its concentration typically varies little within or among fish species (8). The levels of mRNAs for LDH-A and actin were determined as described by Yang and Somero (7). A specific probe for the A-isoform of LDH was used because this is the only isoform of LDH found in white muscle of this species (Linda Z. Holland, Scripps Institution of Oceanography, pers. comm.). A probe for the alpha-isoform of actin was generously provided by Dr. J. Eldridge (National Institutes of Health).

Figure 1 shows the relationship between body mass and each of the biochemical properties measured. Only LDH activity showed a significant dependence on body size, a result in accord with previous studies of many pelagic fishes, including a congener, *Paralabrax clathratus* (1, 3). In both congeners, actin concentration did not scale with body size. However, in *P. clathratus* total protein did scale positively, albeit weakly ($R^2 = 0.36$), with body size, whereas no significant trend was found in *P. nebulifer* (Fig. 1).

The lack of size-related variation in the concentrations of LDH mRNA and actin mRNA suggests that the different scaling patterns for these two proteins are not a simple consequence of different steady-state concentrations of their specific messenger RNAs. Were mRNA levels the primary determinant of protein concentration, then the level of LDH mRNA would rise with increasing body size, in parallel with the observed increase in LDH activity. The lack of correlation between LDH activity and LDH mRNA concentration in muscle of *P. nebulifer* agrees with the results from studies of different tissues of rats (9–10); those studies also failed to show any significant correlation between the two variables. In these particular cases it appears that LDH activity may be regulated by post-transcriptional events. In contrast to these findings, Crawford and Powers (11) reported a strong correlation between the activity of LDH-B and levels of LDH-B mRNA in populations of the fish *Fundulus heteroclitus* from the northern and southern extremes of its distribution range. They hypothesized that the higher enzymatic activities and mRNA levels present in the northern, more cold-adapted population could have resulted from variations in *ldh-b* gene regulation. The mechanisms for regulating enzymatic activity in response to differences in temperature may differ from those that establish the size-dependent patterns in enzyme levels noted in this and earlier studies (1–4). To elucidate the mechanism or mechanisms responsible for size-related variation in LDH activity, as well as for other enzymes that exhibit scaling, it will be necessary to quantify the rates of synthesis and degradation of these enzymes and their mRNAs.

The observation that the total RNA concentration in white muscle of *P. nebulifer* is independent of size was surprising in view of the finding that rates of protein synthesis in fish muscle may change with size and age (12). The RNA pool in a cell is composed primarily of ribosomal and transfer RNAs. Thus, total RNA concentration provides an index of the amount of protein synthetic machinery present in the cell. If the size-independence of total RNA is characteristic of fish white muscle, then size- or age-specific rates of protein synthesis may be governed by factors other than the concentration of the ribosomal protein synthetic machinery present in the cell.

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concentration as a function of body weight. The actin concentration was measured by the DNase I inhibition assay (8). (D) Relative concentration of muscle actin mRNA as a function of body weight. To determine the relative actin mRNA level, an anti-sense RNA probe derived from chicken α-actin was used for the liquid hybridization assay (7). (E) Activity of muscle LDH as a function of body weight. White muscle was homogenized with 10 volumes of 10 m*M* Tris/Cl, pH 7.2 (20°). LDH activity was assayed by adding 20 μl of this homogenate to 2 ml of medium containing 80 m*M* imidazole/Cl buffer (pH 7.0, 20°C), 100 m*M* KCl, 200 μ*M* NADH, and 5 m*M* pyruvate. (F) Relative concentration of muscle LDH mRNA as a function of body weight. The anti-sense RNA probe derived from *Sebastolobus alascanus* LDH-A_4 cDNA was used in liquid hybridization assays (7).

gestions on RNA hybridization assays. The research was supported by National Science Foundation grant IBN92-06660.

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